Recessive Dystrophic Epidermolysis Bullosa

A Review of Disease Pathogenesis and Update on Future Therapies

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ABSTRACT

Objective: Review the pathogenesis of recessive dystrophic epidermolysis bullosa and provide an update on research currently underway that is aimed at treating and potentially curing this severe skin disorder. **Design:** Review article. **Setting:** Private practice and large teaching hospital. **Participants:** None. **Measurements:** N/A. **Results:** Currently, patients with recessive dystrophic epidermolysis bullosa are managed with only supportive care. However, there are several promising new treatment avenues that may help patients in the future. These include gene therapy, cell therapy, and protein-based therapy. Each approach offers distinct advantages and disadvantages. **Conclusions:** The advances in understanding the molecular basis for epidermolysis bullosa over the last few decades has led to significant progress in devising new treatment options. Though many of these approaches remain several years away from regular implementation, it is an exciting time for research in the field. (*J Clin Aesthet Dermatol.* 2015;8(5):41–46.)

Epidermolysis bullosa (EB) refers to a heterogeneous group of inherited mechanobullous disorders caused by mutations in genes that encode structural proteins in the skin. Four major subtypes exist, each characterized by a distinct plane of epidermal-dermal separation following minor trauma. These include EB simplex (EBS) in which skin cleavage occurs within the epidermis, junctional EB (JEB) in which it takes place at the lamina lucida, and dystrophic EB (DEB) where splitting is located just beneath the lamina densa. As of 2007, the reclassified Kindler syndrome, where cleavage occurs at various levels of the skin, is also included. Although it makes up less than five percent of cases of EB, the recessively inherited form of dystrophic EB has been the subject of much research and is the focus of this review.

Recessive dystrophic epidermolysis bullosa (RDEB) is one of the two main subtypes of dystrophic EB, differing from dominant dystrophic epidermolysis bullosa (DDEB) by its recessively inherited pattern. Both forms involve a mutation in the COL7A1 gene, which encodes type VII collagen (C7). This mutation leads to aberrant synthesis of C7 or defective assembly of the protein into anchoring fibrils, resulting in poor epidermal-dermal adherence. Both RDEB and DDEB display further subtypes based on the type of COL7A1 mutation involved, resulting in a wide spectrum of clinical severity (Table 1). In general, DDEB presents with milder phenotypes while RDEB is among the most devastating forms of EB.

Although EB was first defined in 1886,[1] it was not until 1988 that the molecular basis of RDEB began to be understood.[2] In the last two decades, this understanding has led to significant progress in developing promising new therapeutic options for a disease that is currently managed with only supportive care. Research is currently underway for several different treatment strategies that include gene therapy, cell-based therapy, and protein therapy. Principles of each of these approaches will be further explored here.

PATHOGENESIS AND CLINICAL FEATURES

Understanding the molecular basis of the disease is a necessary step in the formulation of targeted therapies. COL7A1 is located on chromosome 3 (3p21.1) and is

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TABLE 1. Subtypes of DDEB and RDEB	
DOMINANT DYSTROPHIC EB	**RECESSIVE DYSTROPHIC EB**
DDEB-generalized	RDEB-generalized severe
DDEB-acral	RDEB-generalized other
DDEB-pretibial	RDEB-inversa
DDEB-pruriginosa	RDEB-pretibial
DDEB-nails only	RDEB-pruriginosa
DDEB-bullous dermolysis of the newborn	RDEB-centripetalis
	RDEB-bullous dermolysis of the newborn

Adapted from Fine JD, Eady RA, Bauer EA, et al. The classification of inherited epidermolysis bullosa (EB): report of the Third International Consensus Meeting on Diagnosis and Classification of EB. *J Am Acad Dermatol.* 2008;58:931–950.

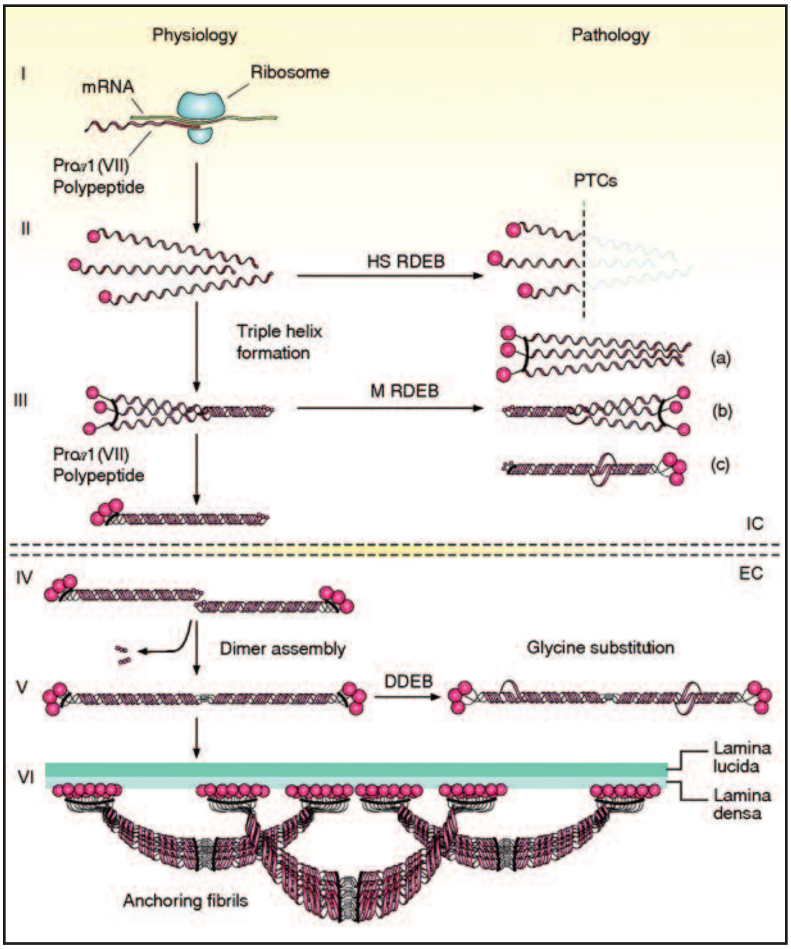

Figure 1. Adapted with permission from Varki R, Sadowski S, Uitto J, et al. Epidermolysis bullosa. II. Type VII collagen mutations and phenotype/genotype correlations in the dystrophic subtypes. *J Med Genet.* 2007;44:181–92. .

transcribed in the nuclei of fibroblasts and keratinocytes. The gene, containing 118 exons, encodes a polypeptide that forms a homotrimer with two identical peptides to make up the C7 molecule.[3] Like all other collagens, these molecules fold into a triple-helical conformation, giving them enhanced stability. They are then secreted into the extracellular matrix where they align in an antiparallel fashion and form dimers. Subsequent aggregation of multiple dimers produces the anchoring fibrils. These fibrils attach to extracellular molecules of the lamina densa in a U-shaped pattern and bind to dermal collagen fibrils to provide structural support between the papillary dermis and overlying basement membrane (Figure 1).

Any inherited predisposition to altered formation of C7 or its anchoring fibrils leads to dystrophic EB. More than 700 different mutations of COL7A1 have been reported.[4] This large number suggests that in many cases, mutations arise *de novo* and are passed on within families, as opposed to a select few mutations evolving over time and being distributed throughout larger populations. Indeed, there is no racial predilection for EB and it occurs in all races worldwide.[5] Given the large number of different COL7A1 mutations, there is a fairly broad spectrum of phenotypes. Table 1 lists the currently recognized subtypes as defined at the 2007 Third International Consensus Meeting on Diagnosis and Classification of EB.[6]

In the autosomal dominant form, a missense mutation occurs on one allele that results in a glycine substitution somewhere along the translated polypeptide. This leads to C7 that does not fold properly. Improper folding in turn leads to impaired secretion of C7 molecules into the extracellular matrix and an altered structure that negatively impacts anchoring fibril formation.[7] Because the allele with the missense mutation is dominantly expressed over the normal allele, patients with this mutation demonstrate disease clinically. Of note is that no specific mutations have been correlated with any of the DDEB subtypes.

In RDEB, missense or nonsense mutations occur on both alleles of COL7A1. Genetic compound heterozygosity is common in these patients as they inherit one type of COL7A1 mutation from one parent and a different type from the other. Combinations of different mutation types expand the possible clinical variations, with the two most common types being RDEB-generalized severe (GS), formerly known as the Hallopeau-Siemens variant, and RDEB-generalized other (GO). RDEB-GS is the most severe and results from two premature termination codon mutations in CO7A1. This leads to abnormally truncated polypeptides unable to form C7. These protein fragments become degraded within the cell leading to absent anchoring fibril production. In RDEB-GO, patients tend to have one allele containing a premature termination codon while the other contains a missense mutation. The missense mutation leads to irregularities in the resulting polypeptide that affects C7 structure. Patients with RDEB-GO usually have a milder phenotype owing to some functional, albeit structurally abnormal, C7.

Interestingly, a recent report of RDEB occurring in monozygotic twins with identical COL7A1 mutations yet very different disease severity highlights that other variables contribute to phenotypic diversity as well. In this case, both twins expressed similar levels of C7, but had variations in several genes within dermal fibroblasts associated with regulation of transforming growth factor-β (TGF-β).[8] The more affected twin had an increased baseline activation of the TGF-β pathway as well as increased expression of interleukin (IL)-6 and MCP-1—proinflammatory cytokines upregulated in fibrotic conditions.[8] TGF-β expression was alternatively decreased in the less affected twin. This example demonstrates the complexity involved with unraveling the molecular pathogenesis of the varying phenotypic manifestations.

Clinically, RDEB-GS patients present with involvement over much of the integument, including the mucous membranes (Figure 2). Esophageal strictures may form, leading to chronic malnutrition and slowed growth, often necessitating gastrostomy tube placement. Progressive scarring leads to fusion of fingers and toes (pseudosyndactyly), loss of nail plates, joint contractures, and eye inflammation with visual impairment. Many patients survive only to their fourth decade as a result of aggressive squamous cell carcinomas (SCC) that arise within areas of repeated scarring. RDEB-GO patients present with many of the same clinical features, but in a milder form (Figure 3). These patients have a slightly better prognosis and median survival of the non-severe subtypes is 55 to 65 years of age.

CURRENT MANAGEMENT

Management of patients with RDEB is presently limited to wound care and attempts to minimize trauma. Avoidance of adhesives and compressive dressings is important as these can induce blister formation. Given their multiple comorbidities, multidisciplinary management is necessary in these patients as well. Common clinical findings include dental caries, esophageal strictures, mitten deformities, nutritional deficiencies, and psychosocial morbidity. Care from a dermatologist, pediatrician, gastroenterologist, nutritional therapist, dentist, and psychologist, among other specialists, is essential. In addition, given the high incidence of developing aggressive SCCs as early as the second decade, regular skin cancer screenings are necessary. Patients should also be forwarded to support groups, such as the dystrophic epidermolyis bullosa research association (DebRA of America: www.debra.org).

Figures 2A and 2B. RDEB-GS. Reprinted with permission from Intong LR, Murrell DF. Inherited epidermolysis bullosa: new diagnostic criteria and classification. *Clin Dermtol.* 2012:30;70–77.

FUTURE THERAPIES

Fortunately, significant progress has been made in the last decade in devising innovative, molecularly based, curative therapies for EB patients. Although regular clinical application still remains years away, it is certainly

Figure 3. RDEB-GO. Authors' photos.

an exciting time for research in the field. The four major treatment strategies being investigated are gene therapy, fibroblast cell therapy, bone marrow stem cell therapy, and protein therapy. Each may be more suitable for certain subtypes than others. Some patients could theoretically benefit from a combination of therapies as well.

Gene therapy. Gene therapy is perhaps the most promising avenue for treatment of RDEB. The general approach is to obtain a sample of an affected patient's skin, harvest the epidermal stem cells, transfer a functional COL7A1 gene into these cells using a viral vector, grow the corrected cells into thin sheets, then graft the sheets over existing wounds. In 2006, this strategy was used successfully to replace the LAMB3 gene in a patient with JEB.[9] The patient was reported to demonstrate a firmly adherent epidermis and lack of blistering for the duration of the one-year follow-up period. Subsequent follow-up over the last six years has demonstrated continued dermal-epidermal stability.[10] RDEB patients are likely to benefit from this method in the future. The downsides are that it is a labor-intensive procedure and the corrected-cell grafts may only be applied to certain areas of the skin. Mucosal sites in particular would be difficult to treat using this approach.

Other experiments have demonstrated success with COL7A1 gene transfer in fibroblasts in murine models.[11,12] Intradermal injection of genetically corrected fibroblasts into mice with RDEB showed that the fibroblasts go on to secrete C7 and form anchoring fibrils. The strategy has potential to treat larger areas of skin. However, questions remain regarding the duration of C7 production by injected fibroblasts. Follow-up studies are still necessary.

Intriguingly, the difficult process of transferring a functional COL7A1 gene into cells may be bypassed in some instances where nature corrects the gene mutation itself. Revertant mosaicism, in which a spontaneous mutation in an affected cell corrects the underlying genetic defect followed by expansion of this cell line, is not rare in EB. It has been reported in up to one-third of JEB patients and also described in RDEB.[13,14] In these cases, culturing the reverted keratinocytes then grafting sheets of these cells onto affected sites can be very beneficial in reducing morbidity and comes with fewer risks compared with other gene transfer methods.[15]

One drawback to the gene therapy approach is that in cases of *ex vivo* gene transfer, there are questions regarding the possibility of developing an immune response to newly produced C7, particularly in patients who are completely deficient of the protein, such as those with RDEB-GS. Since C7 is capable of being immunogenic in some instances, such as in epidermolysis bullosa aquisita (EBA), an immune response to the molecule in C7-naïve patients is theoretically possible and could result in treatment failure. It may be that only patients with some inherent production of C7, however small, benefit. For some, immunosuppression may be necessary. Nevertheless, there is great potential for gene therapy and clinical trials for RDEB patients will likely be underway in the near future.

Fibroblast cell therapy. A therapeutic option perhaps closer to regular clinical application than gene therapy is injection of fibroblasts derived from unaffected donors. A 2008 clinical trial of five patients with RDEB showed that, in three patients, intradermal allogeneic fibroblast injection led to an overall increase in C7 expression.[16] The protein's presence was sustained for several months. While the exact mechanism of C7 production is unclear, the initial thought is that the injected wild-type fibroblasts directly produce C7 themselves. Another possibility, however, is that the fibroblasts elicit a subclinical immune reaction, which leads to production of growth factors that stimulate synthesis of the patient's own mutated C7. The increase in partially functional, rudimentary anchoring fibrils then helps to improve dermal-epidermal adherence. Several observations support this latter mechanism, such as an increase in C7 expression within basal keratinocytes and a better response in patients with more C7 at baseline. If the latter mechanism is correct, only patients with some baseline C7 production and milder disease stand to benefit from this approach. For patients with more severe disease, alternative treatment strategies would be indicated. Follow-up placebo-controlled trials for this method are ongoing.

Bone marrow stem cell therapy. Bone marrow-derived mesenchymal stem cells have also shown potential for treatment of RDEB. These cells are capable of contributing to regeneration of the skin following trauma and have been shown to home-in on areas of tissue damage. In the right microenvironment, the stem cells transition to fibroblasts to bolster wound healing and produce C7. Furthermore, they enhance wound healing by releasing proangiogenic factors, such as vascular endothelial growth factor.[17]

Following successful murine studies, the first clinical trial utilizing bone marrow stem cells was published in 2010.[18] In this study, investigators treated seven children with RDEB using allogeneic bone marrow transplantation from COL7A1+ donors. Patients were noted to have increased C7 expression at the basement membrane and notable clinical improvement following the procedure. A four-year follow-up report revealed sustained improvement.[19] However, the procedure was not without risk as two patients died from complications from the chemoablative preconditioning. Current studies are now investigating bone marrow transplantation with reduced intensity pretransplant chemotherapy.

Other studies are examining the utility of allogeneic mesenchymal stem cells injected intradermally near chronic ulcerations. One report showed there was *de novo* C7 production as well as improved wound healing that lasted several months following this approach.[20] While much work remains to improve the safety and identify the optimal approach for treatment, early studies support the utility of bone marrow stem cells in RDEB patients.

Protein therapy. Successful preliminary studies where investigators injected purified human C7 protein into RDEB mice have led to optimism for use of this strategy in RDEB patients.[21] In the murine models, intradermal injection of C7 resulted in anchoring fibril formation, confirmed with immunogold labeling electron microscopy, and enhanced dermal-epidermal adherence. Interestingly, further experiments have demonstrated that intravenous injection of C7 in mice also improved the RDEB phenotype, the result of C7 molecules homing to areas of the skin in need of repair. The mechanism for this homing is not yet understood, but some suggest C7's inherent structure allows for fixation in the basement membrane zone. The intravenous approach may be helpful in more generalized involvement while intradermal injections may suffice for milder disease. Because the injected protein degrades with time, repeat injections would be necessary, but likely only every 3 to 4 months. As with other models, patients receiving this method of treatment would need to be monitored for development of antibodies to C7. Clinical trials are currently in the enrolling period and are expected to begin in the near future.

SUMMARY

RDEB is among the most severe genodermatoses known, but treatment is currently limited to wound care, avoidance of trauma, active screening for SCC, and general supportive care with a multidisciplinary approach. Alternative therapies are sorely needed. Fortunately, improved understanding of the disease's etiology has led to multiple potential treatment avenues. Ongoing research in these areas, taking place in several countries, offer great hope for the future for patients with this devastating condition.

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